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ORIGINAL ARTICLE

# Hepatic tissue damage induced in *Meriones ungliculatus* due to infection with *Babesia divergens*-infected erythrocytes

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**Abstract** *Babesia divergens* is an intraerythrocytic parasite which is capable of infecting a wide range of vertebrates causing huge economic losses.

Histopathological, hematological and biochemical changes during *B. divergens* infection in female *Meriones ungliculatus* were reported. Animals were challenged with $5 \times 10^6$ *B. divergens*-infected erythrocytes. Parasitemia were maximum at day 5 postinfection where all gerbils died. Infection of gerbils with *Babesia* induced a significant decrease in erythrocytic count as well as the hemoglobin concentration and hematocrit percentage but leucocytes were increased significantly when compared to uninfected gerbils. Liver enzymes aspartate aminotransferase (AST) and aniline aminotransferase (ALT) were significantly increased while albumin and total bilirubin were significantly decreased at day 5 postinfection with *B. divergens*-infected erythrocytes. Histopathological scores of inflammation after infection of gerbils were done using Ischak's activity index and indicated that the liver was severely affected. In conclusion, the study indicated that the course of infection by *B. divergens*-induced alternations in hematology, biochemistry and histopathology of the hepatic tissue.



## 1. Introduction

Babesiosis is due to various tick-borne intraerythrocytic parasites of the protozoan genus *Babesia*. These blood parasites infect many domestic and wild animals throughout the world (Kuttler, 1988). *Babesia divergens* is an intraerythrocytic parasite which is capable of infecting a wide range of vertebrates and it is responsible for important economic losses. It is the most pathogenic and widespread *Babesia* in northern temperate areas (L'Hostis and Chauvin, 1999). Human infections have also been observed, especially in splenectomized patients, and a high mortality rate has been reported (Gorenflot, 1988;

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Spach et al., 1998; Walker et al., 1996). It is believed that the tick responsible for transmission of *B. divergens* to humans is *Ixodes ricinus* (Gorenflot et al., 1998; Telford and Spielman, 1998). Main clinical manifestations of human babesiosis include intravascular hemolysis with hemoglobinuria and jaundice. Persistent high fever, shaking chills, sweating, headache, myalgia, and lumbar and abdominal pain are concomitant symptoms (Marion et al., 2008).

Histological activity index is an accurate analysis of the tissue sections to determine the severity of the pathological change in organs due to infection. Modified quantitative Ishak's scoring was used (Ishak et al., 1995).

The study aims to estimate the histological and biochemical changes induced in hepatic tissue of gerbils due to infection with *B. divergens*.

## 2. Materials and methods

### 2.1. Infection of gerbils

Female *Meriones unguiculatus* aged from 12 to 15 weeks old were used. They were bred under specific pathogen-free conditions in the animal facilities of King Saud University, Riyadh, Saudi Arabia. They were housed in plastic cages and fed on standard diet and given water *ad libitum*. Animals were challenged with $5 \times 10^6$ *B. divergens*-infected erythrocytes. Parasitemia was evaluated in Giemsa stained blood smears from the tail veins (Krücken et al., 2009). The experiments were approved by the state authorities and followed Saudi Arabian law on animal protection.

### 2.2. Hematological and biochemical analysis

The blood was obtained by cardiac puncture, allowed to clot for 30 min at 4 °C, and centrifuged at 3000*g* for 3 min. Sera were collected and stored at −20 °C. Serum aspartate aminotransferase (AST), aniline aminotransferase (ALT), albumin and total bilirubin (Biosystems, Spain) levels were determined using commercial kits on a Shimadzu-UV 1230 model spectrophotometer.

Some blood was collected into tubes with ethylene diamin tetra acetic acid for the determination of some hematological parameters (total erythrocytes count, total Leucocytic count, hemoglobin contents and hematocrit) using an automatic counter (VET-530 CA Medonic; Medonic, Stockholm, Sweden).

### 2.3. Histopathology

Small pieces of liver were fixed in 10% neutral buffered formalin, processed for light microscopic examination, and then 5–7 μm paraffin sections were cut and stained with Hematoxylin and Eosin for histological study. Modified quantitative Ishak scoring system (Ishak et al., 1995) were used; scores of 1–3 were assigned to cases of minimal liver damage, scores of 4–8 to mild, scores of 9–12 to moderate and scores of 13–18 to severe cases.

### 2.4. Statistical analysis

Statistical analyses were performed using an unpaired Student's *t*-test. The data were analyzed by using Excel 2003 (Microsoft, USA), and Sigma Plot 2001 (SPSS, USA).

## 3. Results

### 3.1. Characteristics of B. divergens infection

Gerbils were fully susceptible to *B. divergens*. Challenge with $5 \times 10^6$ parasitized erythrocytes resulted in a lethal outcome of the infection. Parasitemia was about 1% on day one postinfection, then it jumps to 5% on day 2 p.i. The parasitemia rises until reaching about 35% on day 5 p.i. (Fig. 1). No gerbils had been survived after day 5 p.i. (Fig. 1).

Symptoms of babesiosis clearly appeared on day 5 postinfection with shivering, fever and bloody urine. There was a

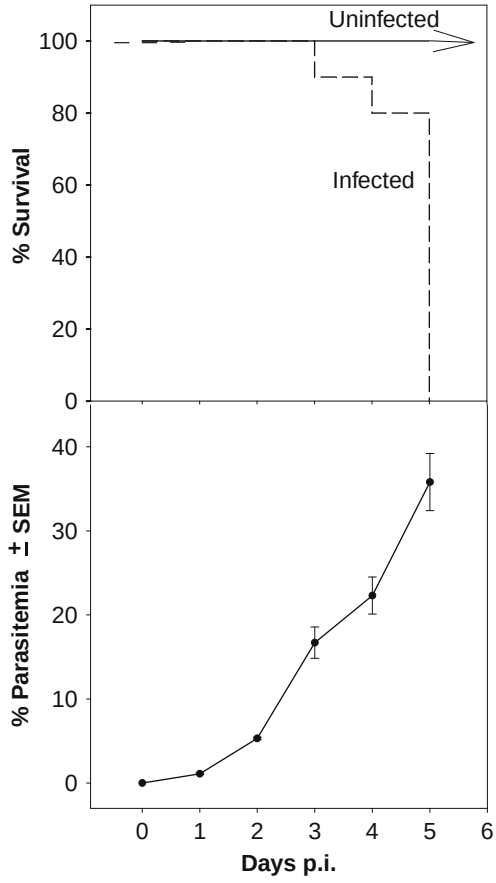


**Figure 1** Characteristics of *B. divergens* infection in *Meriones unguiculatus* challenged with $5 \times 10^6$ parasitized erythrocytes. Parasitemia with survival ($n = 10$). (All values are mean ± SEM.)

**Table 1** Histopathological scores of inflammation after infection of gerbils with *B. divergens*-parasitized erythrocytes.

| Liver parameters | Uninfected gerbils ($n = 5$) | Infected gerbils ($n = 8$) |
|---|---|---|
| Histological activity index[a] | 1–3 | 12–14 |
| Sinusoid dilatation | + | + + + |
| Cytoplasmic vacuolization | + | + + + |
| Binucleated cells | + | + + |
| Cell swelling | No | + + |
| Hyperplasia of Kupffer cells | + | + + + |

[a] Modified according to Ishak et al. (1995). *Score:* 1–3, minimal; 4–8, mild; 9–12, moderate; 13–18, severe.

progressive rise in rectal temperature to approximately 40 °C at death. Animals appeared inactive and anaemic. Anaemia was diagnosed by measuring the erythrocyte counts (2.9 ± $0.4 \times 10^6$/μl), hemoglobin content (5.55 g/dl ± 1.47) and hematocrit (27% ± 1.22) on day 5 p.i., compared with uninfected gerbils which had erythrocytic counts equal to 6.8 ± $0.38 \times 10^6$/μl, hemoglobin content equal to (12.75 g/dl ± 0.87) and hematocrit values equal to 40.6% ± 0.33 (Table 1). Leucocytes were significantly increased ($p < 0.01$) in infected gerbils ($6.9 \times 10^3$/μl ± 0.59) when compared to the uninfected ones ($5.3 \times 10^3$/μl ± 0.71).

### 3.2. Histopathological changes in liver

The liver of infected gerbils undergoes alterations in response to blood-stage *B. divergens*. At maximum parasitemia, the liver became dark-brown and extremely friable, and it was highly edematous with largely dilated. Sinusoids were enriched with macrophages and parasite-containing erythrocytes (Fig. 2A and Table 2). The Kupffer cells are enlarged, and occasionally appear to be in the process of phagocytosis of *B. divergens*-infected erythrocytes (Fig. 2B). Moreover, there is always a strong inflammation in the liver. The lobular inflammation is characterized by predominant infiltrations of lymphocytes, plasma cells, and histiocytes, which are localized in perivascular and parenchymal areas (Fig. 2A). Apoptotic bodies were rare (Fig. 2). All these alterations are considered in the histological liver activity index according to Ishak, which can be categorized as 12–14 for the liver at maximal parasitemia in comparison to 1–3 for non-infected controls (Table 2).

Infected animals suffered impaired liver function. The level of AST and ALT enzymes increased about two folds when compared to the uninfected gerbils (Table 1). Albumin and total bilirubin were significantly decreased due to infection ($p < 0.05$).

**Table 2** Hematological and biochemical analysis of gerbils infected with *B. divergens*-parasitized erythrocytes.

| Items | Uninfected gerbils ($n = 5$) | Infected gerbils ($n = 8$) |
|---|---|---|
| Erythrocytes ($10^6$/mm$^3$) | 6.8 ± 0.38 | 2.9 ± 0.4* |
| Leucocytes ($10^3$/mm$^3$) | 5.3 ± 0.71 | 6.9 ± 0.59* |
| Hemoglobin (g/dl) | 12.75 ± 0.87 | 5.55 ± 1.47* |
| Hematocrit (%) | 40.6 ± 0.33 | 27 ± 1.22* |
| AST (U/l) | 171.6 ± 3.2 | 396 ± 4.3* |
| ALT (U/l) | 41 ± 2.65 | 76.3 ± 3.9* |
| Albumin (g/dl) | 4.43 ± 1.32 | 3.91 ± 1.1* |
| Total bilirubin (mg/dl) | 31.24 ± 2.26 | 11.61 ± 3.52* |

Data presented as mean ± SD. AST, aspartate aminotransferase; ALT, aniline aminotransferase; ALP, alkaline phosphatase.
* $p < 0.05$, significance from the value of control.

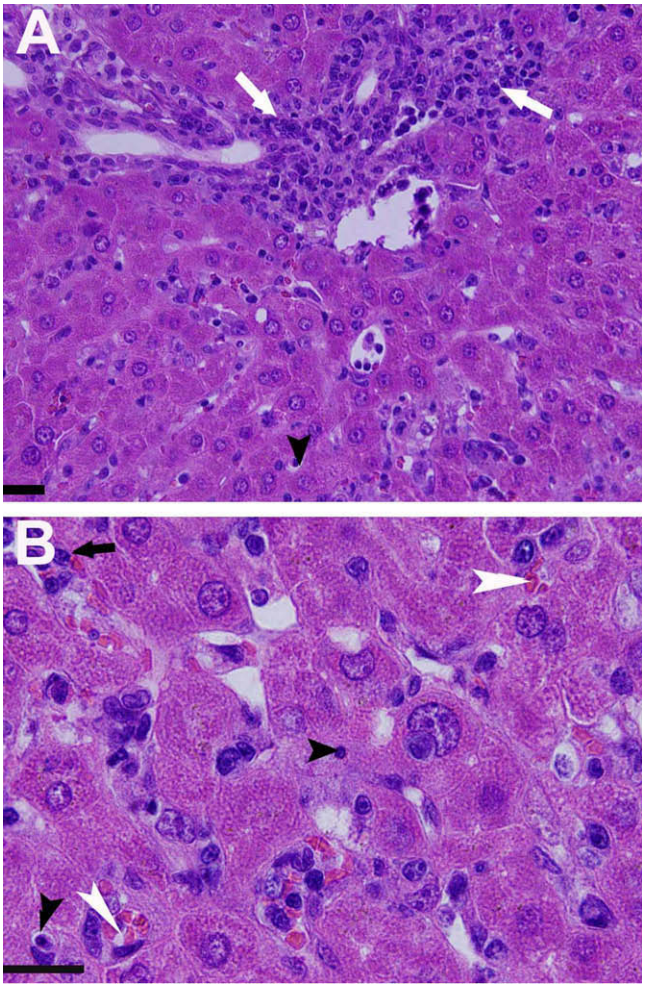


**Figure 2** *B. divergens*-induced changes in liver histology of Gerbils. (A) Lobular necroinflammatory changes in the liver. The inflammatory infiltrate dominated by lymphocytes. (B) *B. divergens*-infected erythrocytes, apoptotic bodies and *B. divergens*-parasitized erythrocytes attached to Kupffer cell. The sections were stained with hematoxylin–eosin. White arrow: inflammatory cells; black arrowheads: apoptotic bodies; black arrow: *B. divergens*-parasitized erythrocyte attached to Kupffer cell; white arrowhead: *B. divergens*-parasitized erythrocyte. Bar = 50 μm.

## 4. Discussion

Gerbils infected with blood-stage parasites are characterized by parasitemia sometimes exceeding 35% of infected erythrocytes and an acute inflammatory response (Schetters et al., in press).

Animals induce immune complex-mediated hepatic tissue lesions similar to those in Falciparum malaria (Wozniak et al., 1997; Krücken et al., 2009).

*Babesia* parasites, like *Thelaria* and malaria parasites, invade erythrocytes of infected animals, resulting in the destruction of parasitized erythrocytes (Otsuka et al., 2002).

The liver plays a central role in babesiosis: it is known as the site where the pre-erythrocytic stages of *Babesia* parasites asexually multiply and where host immune mechanisms develop to fight these pre-erythrocytic stages (Cohen and Lambert, 1982).

Hyperplasia of Kupffer cells were detected in liver sections of infected gerbils, this is due to the need of phagocytosis as a protective mechanism during the course of infection, this result was in agreement with Otsuka et al. (2002) where they studied the immune response of gerbils due to *Babesia rodhaini* infection. Hepatic tissue showed cytoplasmic vacuolation which is mainly a consequence of considerable disturbances in lipid inclusions and fat metabolism occurring under pathological cases (Zhang and Wang, 1984; El-Banhawy et al., 1993).

The decrease in erythrocytic count, hemoglobin, hematocrit, increase in leucocyte counts might be due to severity of infection. Increases in serum AST and ALT activities depending on hepatocellular damage (San Martin-Nunz et al., 1988). Decreases in the albumin levels were attributed to the decrease of albumin production resulting from hepatic degeneration associated with infection (Yeruham et al., 1998).

Although anaemia is the major symptom and cause of mortality in animals with *Babesia* infection, the pathogenesis of the anaemia remains unclear. In general, *Babesia* parasites, like *Theileria* and malaria parasites, invade erythrocytes of infected animals, resulting in the destruction of the parasitized erythrocytes (Kawamura et al., 1987).

The results of the present study indicated that the course of the infection induced by *B. divergens* might be determined by alterations in clinical, hematological, biochemical and pathological findings.

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## References

Cohen, S., Lambert, P.H., 1982. In: Cohen, S., Warren, K.S. (Eds.), Immunology of Parasitic Infections, second ed. Blackwell Scientific Publications, Oxford, pp. 422–474.

El-Banhawy, M.A., Sanad, S.M., Sakr, S.A., El-Elaimy, I.A., Mahran, H.A., 1993. Histopathological studies on the effect of the anticoagulant rodenticide "Brodifacoum" on the liver of rat. J. Egypt Ger. Soc. Zool. 12, 185–227.

Gorenflot, A., Moubri, K., Precigout, E., Carcy, B., Schetters, T.P., 1998. Human babesiosis. Ann. Trop. Med. Parasitol. 92, 489–501.

Gorenflot, A., 1988. La babesiose humaine en Europe. Lett. lnfect. 3, 345–347.

Ishak, K., Baptista, A., Bianchi, L., Callea, F., Degroote, J., Gudat, F., Denk, H., Desmet, V., Korb, G., Macsween, R.N.M., Phillips, M.J., Portmann, B.G., Poulsen, H., Scheuer, P.J., Schmid, M., Thaler, H., 1995. Histological grading and staging of chronic hepatitis. J. Hepatol. 22, 696–699.

Kawamura, M., Maede, Y., Namioka, S., 1987. Mitogenic responsibilities of lymphocytes in canine babesosis and the effects of splenectomy on it. Jpn. Vet. Res. 35, 1–10.

Krücken, J., Delic, D., Pauen, H., Woytalla, A., El-Khadragy, M., Dkhil, M.A., Mossmann, H., Wunderlich, F., 2009. Augmented particle trapping and attenuated inflammation in the liver by protective vaccination against *Plasmodium chabaudi* malaria. Malar. J. 8, 54–67.

Kuttler, K.L., 1988. World-wide impact of babesiosis. In: Ristic, M. (Ed.), Babesiosis of Domestic Animals and Man. CRC Press, Inc., Boca Raton, Fla., pp. 1–22.

L'Hostis, M., Chauvin, A., 1999. *Babesia divergens* in France: descriptive and analytical epidemiology. Parassitologia 41, 59–62.

Marion, B., Melanie, N., Michaela, K., Gerold, S., Julia, W., 2008. *Babesia* species occurring in Austrian *Ixodes ricinus* ticks. Appl. Environ. Microbiol. 74, 4841–4846.

Otsuka, Y., Amasaki, M., Yamato, O., Maede, Y., 2002. The effect of macrophages on the erythrocyte oxidative damage and the pathogenesis of anemia in *Babesia gibsoni*-infected dogs with low parasitemia. J. Vet. Med. Sci. 64, 221–226.

San Martin-Nunz, B.V., Ordonz-Escudero, D., Alunda, J.M., 1988. Preventive treatment of rabbit coccidiosis with a-difluoromethylornithine. Vet. Parasitol. 30, 1–10.

Schetters, T.P., Kleuskens, J.A., Van De Crommert, J., De Leeuw, P.W., Finizio, A.L., Gorenflot, A., 2009. Systemic inflammatory responses in dogs experimentally infected with *Babesia canis*; a haematological study. Vet. Parasitol. 162, 7–15.

Spach, D.H., Liles, W.C., Campbell, G.L., Quick, R.E., Anderson, D.E., Fritsche, T.R., 1998. Tick-borne disease in the United State. N. Engl. J. Med. 329, 936–947.

Telford, S.R., Spielman, A., 1998. Babesiosis of humans. In: Collier, L., Balows, A., Sussman, M. (Eds.), Topley and Wilson's Microbiology and Microbial Infections, ninth ed., vol. 5. Arnold, London, England, pp. 349–359.

Walker, D.H., Barbour, A.G., Oliver, J.H., 1996. Emerging bacterial zoonotic and vector-borne diseases. Ecological and epidiological factors. JAMA 275, 463–466.

Wozniak, E.J., Barr, B.C., Thomford, J.W., Yamane, I., McDonough, S.P., Moore, P.F., Naydan, D., Robinson, T.W., Conrad, P.A., 1997. Clinical, anatomic, and immunopathologic characterization of *Babesia gibsoni* infection in the domestic dog (*Canis familiaris*). J. Parasitol. 83, 692–699.

Yeruham, I., Hadani, A., Galker, F., Avidar, Y., Bogin, E., 1998. Clinical, clinico-pathological and serological studies of *Babesia ovis* in experimentally infected sheep. Zentralbl. Veterinarmed. 45, 385–394.

Zhang, L.Y., Wang, C.X., 1984. Histopathological and histochemical studies on the toxic effect of brodifacoum in mouse liver. Acta Acad. Med. Sci. 6, 386–388.